



Morphological and antioxidant impairments in the spinal cord of male offspring rats following exposure to a continuous 900 MHz electromagnetic field during early and mid-adolescence



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ABSTRACT

The effects of devices emitting electromagnetic field (EMF) on human health have become the subject of intense research among scientists due to the rapid increase in their use. Children and adolescents are particularly attracted to the use of devices emitting EMF, such as mobile phones. The aim of this study was therefore to investigate changes in the spinal cords of male rat pups exposed to the effect of 900 MHz EMF. The study began with 24 Sprague-Dawley male rats aged 3 weeks. Three groups containing equal numbers of rats were established—control group (CG), sham group (SG) and EMF group (EMFG). EMFG rats were placed inside an EMF cage every day between postnatal days (PD) 21 and 46 and exposed to the effect of 900 MHz EMF for 1 h. SG rats were kept in the EMF cage for 1 h without being exposed to the effect of EMF. At the end of the study, the spinal cords in the upper thoracic region of all rats were removed. Tissues were collected for biochemistry, light microscopy (LM) and transmission electron microscopic (TEM) examination. Biochemistry results revealed significantly increased malondialdehyde and glutathione levels in EMFG compared to CG and SG, while SG and EMFG catalase and superoxide dismutase levels were significantly higher than those in CG. In EMFG, LM revealed atrophy in the spinal cord, vacuolization, myelin thickening and irregularities in the perikarya. TEM revealed marked loss of myelin sheath integrity and invagination into the axon and broad vacuoles in axoplasm. The study results show that biochemical alterations and pathological changes may occur in the spinal cords of male rats following exposure to 900 MHz EMF for 1 h a day on PD 21–46.

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1. Introduction

During the course of their daily lives, human beings employ many technological devices (including microwaves, mobile phones, computers, magnetic resonance imaging equipment, radios, base stations and Wi Fi) that not only make their lives

easier but also expose them to the effects of electromagnetic field (EMF) (Consales et al., 2012). Moreover, a rapid rise is taking place in the use of mobile phones, the most important verbal communication devices today, and as the age at which individuals begin using these tools decreases, they are becoming more and more prevalent among children and young people. This has resulted in debate and concerns over the effects of human health on exposure to EMF emitted by mobile phones (Leitgeb, 2008).

Studies report that long-term exposure to EMF increases blood-brain permeability (Salford et al., 1994; Schirmacher et al., 2000).

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Results from these studies suggest that EMF can lead to morphological and biochemical changes in central nervous system (CNS) tissues, such as the brain, cerebellum and spinal cord. Findings from other studies investigating the relation between EMF and CNS structures also support this hypothesis. For example, EMF has been reported to cause a decrease in cerebellar Purkinje cell numbers (Sonmez et al., 2010) and changes in the electrophysiology of Purkinje cells (Haghani et al., 2013). EMF has also been reported to cause a decrease in pyramidal cells in the brain (Odaci et al., 2008; Bas et al., 2009a,b; Baş et al., 2013) and various histopathological changes in the spinal cord (Odaci et al., 2013). However, very few of these studies have investigated the effect of EMF on the spinal cord at the cellular level.

The question of whether 900 MHz EMF causes oxidative stress in the spinal cords of adolescents is one that needs to be addressed. Oxidative stress occurs following increase of free radicals in tissues, and causes damage in such important structures as cell lipid, DNA, protein and carbohydrate (Johansen et al., 2005). Free radicals affect membrane lipids, and peroxidation occurs as a result of reactions they cause in the cell membrane. Cellular protection following oxidative stress is provided by antioxidants and antioxidant defense systems that implies a high probability of the levels of some antioxidants and antioxidative system enzymes in tissue increasing or decreasing. Malondialdehyde (MDA), a marker of peroxidative injury, emerges as a result (Cheeseman and Slater, 1993). This study therefore investigated MDA, glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) levels in the spinal cord as markers of oxidative stress, since EMF is one of the main causes of oxidative stress in tissues (Uzar et al., 2011; Consales et al., 2012; Nazıroğlu et al., 2012; Kiray et al., 2013).

Adolescence in rodents lasts from postnatal days (PD) 21 to 60 and consists of early adolescence (prepubescent animals, from PD 21 to 34), mid-adolescence (periadolescent, from PD 34 to 46) and late adolescence (young adult, from PD 46 to 59) (Tirelli et al., 2003). Our study was planned to include both early and late adolescence. Our review of the literature revealed several studies investigating the effects of EMF of varying durations and intensities on other tissues, particularly CNS structures such as the brain and cerebellum. However, we identified no studies of changes in the spinal cord in male rats exposed to the effect of 900 MHz EMF in early and middle adolescence. Since the spinal cord is the basic pathway for information connecting the brain to the peripheral nervous system, pathologies that may occur in the spinal cord may lead to behavioral changes by causing problems in the exchange of information between the brain and the peripheral nervous system. Our aim was therefore, to investigate changes in the spinal cords of male rats exposed to continuous 900 MHz EMF for 1 h a day in early and middle adolescence using biochemical and microscopic analysis techniques.

2. Materials and Methods

2.1. Animals and study groups

Twenty-four Sprague-Dawley male rats (21 days old, weighing 28–45 g) were obtained from the Experiment Animals Surgical Research and Application Center of Karadeniz Technical University (Trabzon, Turkey) (ESRACKTU). All experiments and procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. Ethical approval was obtained from the KTU Animal Care and Ethics Committee. Rats were housed in stainless steel cages in a pathogen-free environment in the ESRACKTU in a thermoregulator-controlled room at $22 \pm 1^\circ\text{C}$ under a 12 h light/12 h dark cycle, and in $50\% \pm 10$ humidity except during EMF and sham exposure. Animals were allowed free access to tap water and commercial standard rat chow. Rats were equally divided into three groups—control group (CG), sham group (SG) and EMF group (EMFG). EMFG rats were exposed to the effect of 900 MHz EMF inside an EMF cage for 25 days from PD 21 onward for 1 h a day (at the same time, 11.00 a.m.–12.00 p.m., each day). SG rats were placed inside the EMF cage under the same conditions and for the same length of time (10.00 a.m.–11.00 a.m., each day). These were not exposed to the effect of EMF. Before SG rats were placed inside the cage, EMF was measured inside the

cage, outside the cage and randomly in different areas of the EMF room, using a broadband EMF measuring device (CA 43 Isotropic Electrical Field Intensity Meter, Chauvin Arnoux Group, Paris, France). Once it had been established that no EMF was present, rats were placed inside the cage and exposed to a sham effect. EMF and sham procedures were concluded on PD 46. No procedure was performed on CG rats, which were allowed to feed freely inside their cages throughout the experimental period.

2.2. EMF application room and EMF exposure system

A special EMF exposure room, $2.5 \times 3 \text{ m}^2$ in size, was prepared in the ESRACKTU in order to expose EMFG rats to EMF and SG rats to sham procedures. This chamber contained nothing but the EMF exposure system and was used for nothing except for EMF and sham procedures. Apart from for EMF and sham procedures, rats from all groups were kept in a room inside the ESRACKTU and under conditions described in the Study Design and Animals section. Rats were exposed to EMF or sham procedures by being placed inside a $30 \text{ cm} \times 42 \text{ cm} \times 50 \text{ cm}$ Plexiglas cage within the EMF exposure system, referred to as the EMF cage. This cage was specially manufactured for use in our EMF experiments and had a floor area sufficiently large for the rats to move about freely (126 cm^2 floor area). An ultra-high frequency oscillator (1218-BV, Lockable Oscillator, 900–2000 MHz, General Radio Company, Concord, Massachusetts, USA, Serial No. 1483) with output power of approximately 300 mW and frequency adjusted to 900 MHz was used to establish a 900 MHz EMF inside the cage. The oscillator was attached to a continuous fixed power source (1267-B Regulated Power Supply, General Radio Company, Concord Massachusetts, USA, Serial No. 903) to allow the oscillator to function and to meet the need for continuous energy. The oscillator outlet was attached via a coaxial cable to a half wave dipole antenna made from a copper rod, 15 cm in length and 1 mm in diameter. The dipole antenna was suspended 11 cm inside the cage from the open upper surface and installed in the central part of the cage (Fig. 1). EMFG rats were placed inside the EMF cage as part of the system designed as described and exposed to 900 MHz EMF for 1 h a day at the same time every day.

2.3. Electromagnetic field intensity measurements

In order to determine the distribution of the EMF intensity to which the rats were exposed, EMF was measured from nine different points on the floor of the EMF cage (Fig. 1) during the EMF procedure with rats inside the cage and with the cage empty, from the bottom surface of the cage and from inside it. These measurements were performed using a broad band electric field measuring device (CA 43 Isotropic Electrical Field Intensity Meter, Chauvin Arnoux Group, Paris, France) with a 100 kHz–2.5 GHz measurement interval. On the basis of measurements throughout the study with the cage empty, positional mean EMF intensity was 10.6 V/m beneath the cage and 7.1 V/m inside it. The positional mean EMF intensity with rats inside the cage was 8.9 V/m beneath the cage and 7.3 V/m inside it (Table 1). The whole-body specific absorption rate (SAR) was calculated at 0.01 W/kg (RadHaz SAR Equivalenc Calculator Version 1.0, © 2000 Richard Tell Associates, Inc., Mesquite, NV, USA). According to these measurements, rats were exposed to a mean electrical field intensity of 7.3 V/m inside the cage (0.21 W/m^2). Although our continuous 900 MHz EMF exposure system does not completely represent the effects of mobile phones etc. on humans, this value is regarded as equivalent to the

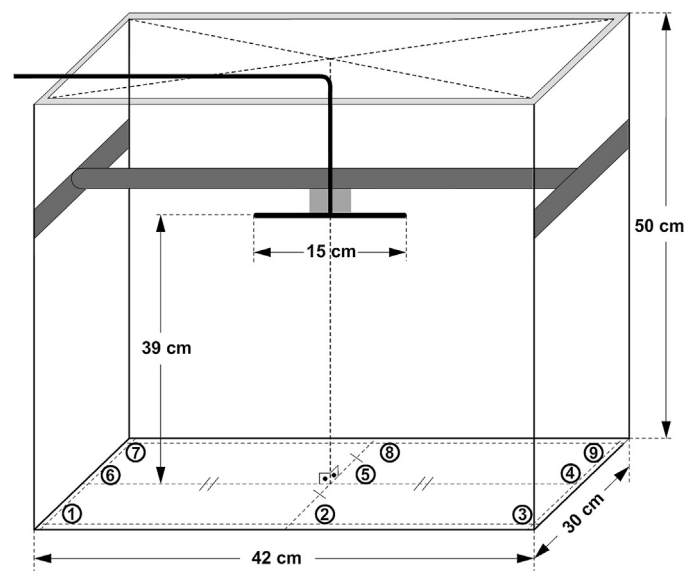


Fig. 1. Dimensions of the Plexiglas cage used to expose rats to EMF and sham procedures. The positions of the nine point used to measure EMF intensity on the floor of the cage are also shown.

Table 1

Electromagnetic field values measured from beneath and inside the Plexiglas cage from nine different points, when the cage was empty and during application of EMF.

Measurements site	Electromagnetic field measurement values (V/m)			
	Cage empty		Male rats inside the cage	
	Beneath cage	Inside cage	Beneath cage	Inside cage
1	7.3	8.3	6.9	5.6
2	8.1	7.7	9.8	7.2
3	7	6.9	9.7	6.5
4	11.3	6.1	8.1	7.4
5	13	9.2	8.4	8.1
6	12.3	6.1	9.2	7.8
7	13.6	5.7	10.9	7.1
8	12.1	7.9	8.6	8.4
9	11.3	6.5	8.92	8.2
Mean $\pm$ SD	10.6 $\pm$ 2.5	7.1 $\pm$ 1.1	8.9 $\pm$ 1.1	7.3 $\pm$ 0.8

intensity of EMF that mobile phones in speak mode can create in their immediate surroundings (mean 1–10 V/m for variables, i.e., mobile phone model and distance from the base station). These values are the limit values set out for a single source in GSM-900 base station systems (Baş et al., 2013; Hancı et al., 2013; İkinci et al., 2013; Odacı et al., 2013, 2015; Topal et al., 2015; Türedi et al., 2015).

2.4. Surgical procedures

One day after the final day of exposure to EMF (PD 47), all rats from all groups were sacrificed on the same day by decapitation under deep anesthesia (Ketalar® 50 mg/kg, Eczacıbaşı Co., Istanbul, Turkey) under sterile conditions. The upper thoracic region of all spinal cords was then removed. Each spinal cord was divided into three parts for biochemical, light microscopic (LM) and transmission electron microscopic (TEM) analyses.

2.5. Light microscopic procedures

Spinal cords for collected LM investigation were subjected to routine histological preparation procedures. First they were placed in 10% formaldehyde for fixation. Following storage in formaldehyde for 1 day, spinal cords were subjected to decalcification. Spinal cords were placed into a 6% solution of trichloroacetic acid (Sigma-Aldrich, Cat. No. T6399) in distilled water for 4–5 days at 4 °C. After decalcification in 6% trichloroacetic acid, the spinal cords were washed in phosphate buffered saline for 20 min. They were then held under tap water overnight before being fixed in paraffin and sliced into sections 7 μ m thick using a fully automatic microtome (Leica RM 2255, Leica Instruments, Nussloch, Germany) and stained with cresyl violet. Histopathological examination was performed under a research microscope (Olympus, BX51, Japan), and photographs were taken using a DP71 camera attachment (DP71, Olympus, Japan).

2.6. Transmission electron microscopic procedures

Spinal cords collected for TEM investigation were trimmed to a volume of 1 mm³. The trimmed parts were fixed for 1.5 h in phosphate buffer containing 2.5% glutaraldehyde at pH 7.3. Post-fixation was then performed for 1 h in a solution of the same buffer with 1% OsO₄. Specimens were rinsed in the buffer and dehydrated through increasing degrees of alcohol. Blocks were made transparent in propylene and fixed in araldite (Araldite CY212, Agar Scientific, Essex, UK). Blocks were then kept in a stove. Sections 450 nm in thickness were taken from the blocks using an ultramicrotome (LKB Nova, Bromma, Sweden), placed on glass slides and stained with Toluidine blue (Sigma-Aldrich Co. LLC., St. Louis, USA). Ultrathin sections 30–40 nm thick were taken from specific areas of semi-thin sections and stained with uranyl acetate and Reynold's lead citrate for TEM analyses. TEM images were obtained using a JEOL 100SX TEM (JEOL Ltd., Akishima, Tokyo, Japan) and photographed (Kodak 4489, Eastman Kodak Company, Rochester, NY).

2.7. Biochemical analysis

Levels of SOD (Sun et al., 1988) and CAT (Aebi, 1974) enzyme activities and levels of GSH (Sedlak and Lindsay, 1968) and lipid peroxidation (LPO) were determined in the spinal cord tissues collected for biochemical tissue analyses. One third of the spinal cord tissues in tubes stored at –80 °C in a deep freezer (Ultra-Low Temperature Laboratory Freezers U410 Premium, New Brunswick Scientific Co. Inc., England) were used for this aim. Decomposition of H₂O₂ in the presence of CAT enzyme activities was observed at 240 nm. The amount of enzyme required to decompose 1 mmol of H₂O₂ per minute, at 25 °C and at pH 7.8 was described as CAT activity. Results were expressed as mmol/min/mg of spinal cord tissue (Aebi, 1974). Tissues were ground with liquid nitrogen in a mortar. Next, 0.5 g tissue samples were treated with 4.5 ml of buffer (SOD, 0.2 mM Tris-HCl buffer, pH 7.4; CAT,

50 mM phosphate buffer, pH 7; GSH, 50 mM Tris-HCl buffer, pH 7.4; LPO, 10% KCl solution). The mixtures were homogenized on ice for 15 min using a homogenizer (Ultra Turrax T25, Rose Scientific Ltd., Edmonton, Canada). Homogenates were filtered and centrifuged for 900 $\times$ g (10 min at 4 °C). The resulting supernatants were employed for biochemical measurements, all of which involved a spectrophotometer (Shimadzu Co., UV-vis Spectrophotometer, UV-1601, Japan). Estimations of SOD enzyme activities were based on the generation of superoxide radicals produced by xanthine and xanthine oxidase. SOD activity was measured at 560 nm by the degree of inhibition of this reaction. Activity was expressed as mmol/min/mg spinal cord tissue (Sun et al., 1988). The amount of GSH in the spinal cord tissues was calculated using 5, 5'-dithiobis (2-nitrobenzoic acid) according to the modifying method described by Sedlak and Lindsay (1968). The absorbance was measured at 412 nm, and tissue GSH levels were expressed as nmol/mg spinal cord tissue. The level of LPO in tissues was determined by estimating MDA using the thiobarbituric acid test (Ohkawa et al., 1979).

2.8. Statistical analysis

Normal distribution of data was assessed using the Shapiro–Wilk test. Since data were not normally distributed, comparisons between SOD, CAT, GSH and MDA levels were performed using the Kruskal–Wallis test. As described by others, nonparametric tests must be employed when variances in a population are unequal (Tunc et al., 2007). We therefore used the nonparametric Mann–Whitney *U* test to compare biochemical parameters between groups. Significance of mean values was set at *p* < 0.05. All statistical analyses were performed on SPSS software (Statistical Package for the Social Sciences, version 13.0, SPSS Inc., Chicago, IL, USA). All data were expressed as means $\pm$ SEM for each group.

3. Results

3.1. Light microscopic observations

At LM evaluation of CG (Fig. 2A) and SG (Fig. 2B), spinal cords exhibited a normal, healthy appearance. Neurons also exhibited a normal morphological appearance. Histopathological evaluation of the EMFG under LM revealed atrophy and an irregular appearance in the spinal cord, increased vacuolization, myelin thickening and irregularity and mild infiltration in the perikarya (Fig. 2C and D).

3.2. Transmission electron microscopic observations

Axon and myelin sheath fine structures were normal in sections from CG (Fig. 3A). In CG, type II ganglia in the spinal cord exhibited a typical appearance (Fig. 3B). Under TEM, sections from EMFG exhibited marked compromise of the internal and external myelin sheaths, and the internal sheath had invaded the axoplasm. Widespread losses were observed in axon cytoplasm, and wide vacuoles were present in these areas. Additionally, electron-dense particles were observed in cytoplasm (Fig. 3C). Examination of SG sections under TEM revealed that the axon and myelin sheath had a typical fine structure similar to that of CG (Fig. 3D). The most noteworthy TEM finding in EMFG, in contrast to CG and SG, was invasion into the axon through marked loss of myelin sheath integrity, together with wide vacuoles causing losses in axoplasm (Fig. 3C).

3.3. Biochemical analysis results

MDA levels in SG were lower than those in CG (*p* = 0.004), while EMFG MDA levels were significantly higher than those of both SG (*p* = 0.004) and CG (*p* = 0.004). CAT levels were significantly higher in SG (*p* = 0.004) and EMFG (*p* = 0.004) compared to CG. In addition, SOD levels in SG increased significantly compared to CG *p* = 0.003), and SOD levels in EMFG also increased significantly compared to CG (*p* = 0.013). Furthermore, EMFG levels were significantly higher compared to those of CG and (*p* = 0.004) SG (*p* = 0.004) (Table 2).

4. Discussion

The effects of EMF on living tissues has become a subject of intensive research parallel to the increasing use of mobile phones

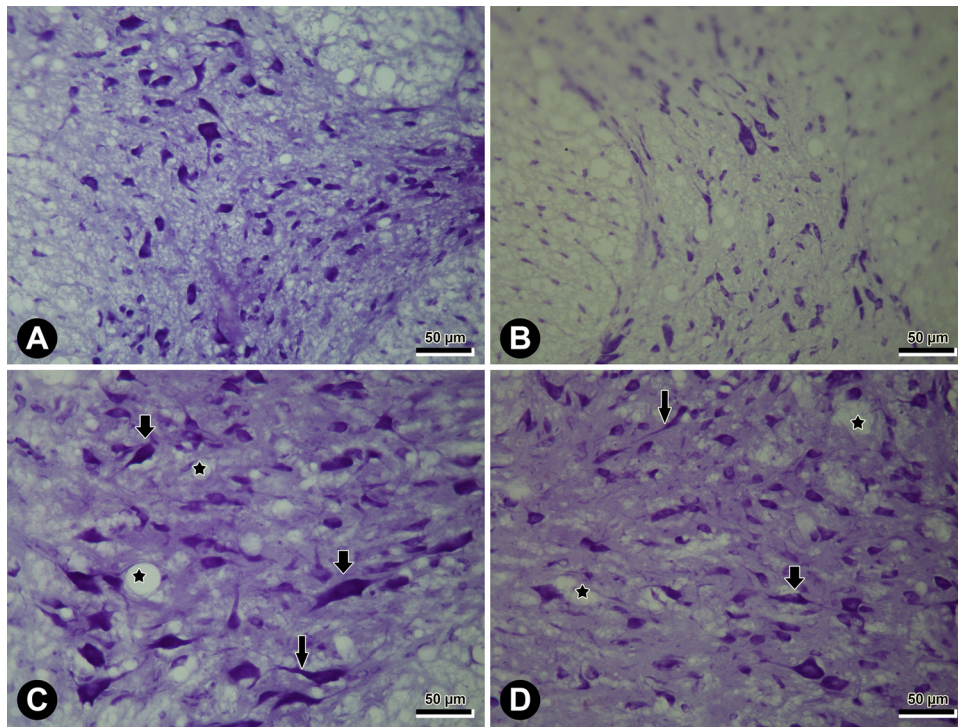


Fig. 2. Spinal cords from CG, SG and EMFG under light microscopy. Gray and white matter with a normal appearance and neurons with normal morphological architecture can be seen in CG (A) and SG (B). In EMFG, however, the images show increased vacuolization in the spinal cord (star), myelin thickening (arrow), irregularity in the perikaryon (thick arrow) and mild infiltration (C and D). H&E $\times 400$.

and the ever lower ages at which such use begins (Odaci et al., 2008; Sonmez et al., 2010; Uzar et al., 2011; Consales et al., 2012; Haghani et al., 2013; Baş et al., 2013). An increase in the numbers of studies showing that these effects may cause various reversible and irreversible pathologies has led to more serious questioning of the effects of mobile phones on human health (Corle et al., 2012; Davis et al., 2013). Compared with adults, children and babies are normally considered to be more sensitive to exposure to physical,

chemical or biological agents. At higher frequencies, children absorb more energy from external EMFs than adults because their bodies are smaller than those of adults and their tissues normally contain a greater number of ions and thus have higher conductivity (IEGMP, 2000; van Rongen et al., 2004).

In 2000, the UK Independent Expert Group on Mobile Phones (IEGMP) published a report to examine the potential effects of mobile phones, base stations and transmitters on health. That report

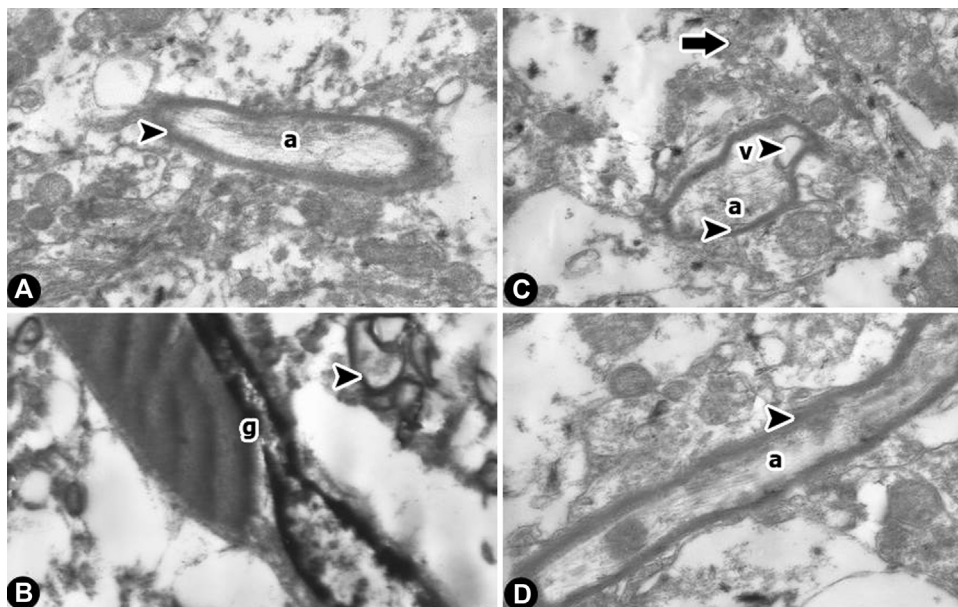


Fig. 3. TEM images from CG, SG and EMFG. (A) The myelin sheath (arrowhead) and axon (a) in the spinal cord in CG exhibit normal morphology. $\times 10,000$. (B) In addition to a normal myelin sheath (arrow head) in the TEM image of a spinal cord from CG, a normal type II ganglion cell (g) can also be seen. $\times 8000$. (C) TEM image of injured spinal cord from EMFG. Losses can be seen in the internal and external layers of the myelin sheath, with the inner layer invading the axon (a) (arrow head). Wide losses in cytoplasm are replaced by wide vacuoles (v). Electron-dense particles can also be seen in cytoplasm (arrow). $\times 15,000$. (D) Normal myelin sheath (arrow head) and axon (a) in spinal cord from SG. $\times 15,000$. Uranyl acetate and lead citrate staining.

Table 2

Biochemical analysis results for medulla spinalis tissues from CG, SG and EMFG rats ($n=8$ for each group).

Biochemical parameters	CG	SG	EMFG
MDA (nmol/mg tissue)	27.78 $\pm$ 0.44	22.71 $\pm$ 0.31 ^a	47.06 $\pm$ 0.29 ^b
CAT (mmol/min/mg tissue)	0.11 $\pm$ 0.07	0.31 $\pm$ 0.01 ^c	0.37 $\pm$ 0.02 ^d
SOD (mmol/min/mg tissue)	0.01 $\pm$ 0.01	2.12 $\pm$ 0.14 ^c	1.52 $\pm$ 0.34 ^d
GSH (nmol/mg tissue)	0.73 $\pm$ 0.04	0.71 $\pm$ 0.01	1.29 $\pm$ 0.06 ^b

The data represent mean $\pm$ SEM.

^a SG MDA levels decreased significantly compared to CG ($p=0.004$).

^b EMFG MDA and GSH levels increased significantly compared to SG and CG ($p=0.004$ and $p=0.004$, respectively).

^c SG CAT and SOD levels increased significantly compared to CG ($p=0.004$ and $p=0.003$, respectively).

^d EMFG CAT and SOD levels increased significantly compared to CG ($p=0.004$ and $p=0.013$, respectively).

says in paragraph 6.63 that; “There is evidence that at the frequencies used in mobile phone technology, children will absorb more energy per kilogram of body weight from an external electromagnetic field than adults (see paragraph 4.37). A one year old could absorb around double, and a five year old around 60%, more than an adult. Additionally, since children are being exposed to RF radiation from base stations (and from mobile phones) from a younger age than adults, they will have a longer time in which to accumulate exposure over the course of their lives, and a longer time for any delayed effects of exposure to develop (IEGMPE, 2000)”.

Therefore, warnings concerning the use of mobile phones, particularly by children, have been issued by several countries. The Belgian Ministry of Public Health forbids the selling of mobile phones to children under 7, and advertisements for such devices are prohibited during TV programs aimed at children. The Australian federal government produced a fact sheet concerning methods for reducing exposure to wireless devices, such as mobile phones. It also suggests that parents restrict their children's exposure to them. France is also banning the advertising of mobile phones to children under 12 (Morgan et al., 2014). However, debates regarding the effects of EMF emitted by mobile phones on living tissues and organs are still continuing. Mobile phones are attached to the belt and carried close to the lower back in many countries. It is therefore inevitable that signals reaching the mobile phone will affect organs in the back, such as the spinal cord, and organs in the region. This represented one of the hypotheses of this study. The aim of this study was therefore to investigate the effect of 900 MHz EMF on the spinal cord of rats in adolescence.

Previous studies have reported that oxidative stress can occur in tissues for various reasons, and that EMF may be one of the most important of these causes (Köylü et al., 2006; Uzar et al., 2011; Consales et al., 2012; Hancı et al., 2013; Kiray et al., 2013; Odacı et al., 2015). For example, Uzar et al. (2011) reported that oxidative stress developed in the spinal cords of rats in the prenatal and postnatal periods following the application of 50 MHz. However, the number of studies investigating the oxidative stress that 900 MHz EMF applied to rats in adolescence may cause in the spinal cord is negligible. Glutathione is a major component of the antioxidant system that defends cells (i.e., neurons) against the toxic effects of reactive oxygen/nitrogen species (Pehar et al., 2015), and systemic administration of bioavailable SOD/CAT mimetics protects against neuronal cell death (Peng et al., 2005). Our study therefore examined levels of the oxidative stress markers MDA, GSH, CAT and SOD in the spinal cord. The study results showed that MDA and GSH levels in EMFG increased significantly compared to those in CG and SG, while CAT and SOD levels decreased compared to CG. This shows that 900 MHz EMF applied continuously for 1 h a day can lead to oxidative stress. In addition, MDA levels in SG decreased significantly compared to those in CG, while CAT and SOD levels increased. Although this

shows that EMF applied for 1 h increased oxidative stress, it also suggests that keeping rats inside the EMF cage for 1 h without exposing them to EMF also led to oxidative stress. We do not therefore think that it is possible to conclude, solely on the basis of the results of this study that 900 MHz EMF applied for 1 h leads to oxidative stress in the spinal cord of rats in adolescence.

This study also assessed the effect of 900 MHz EMF on the spinal cord at the TEM and LM levels in terms of histopathology. At TEM evaluation axon invagination through significant loss of myelin sheath integrity and wide vacuoles in axoplasm were determined in EMFG. LM evaluation revealed atrophy, increased vacuolization, myelin thickening and infiltration and irregularities in the perikarya. The presence of pathological changes at both the TEM and LM levels shows that EMF applied in adolescence also impairs the morphology of the spinal cord. These findings are comparable both with those of previous studies of ours and by others investigating the effects of 900 MHz EMF on other tissues (including the testis, kidney, spleen, thymus, liver and heart) (Hancı et al., 2013; 2015; Odacı et al., 2015; Odacı and Özyılmaz, 2015; Topal et al., 2015; Türedi et al., 2015). For example, in our study of the effect of 900 MHz EMF on spleen and thymus tissue we determined significant histopathological changes at the TEM and LM levels (Hancı et al., 2015).

In another study of ours, pregnant rats were exposed to 900 MHz EMF for 1 h every day daily between days 13 and 21 of pregnancy. Female rat pups' upper thoracic spinal cords were removed on PD 32 and examined under LM (Odacı et al., 2013). Histopathological evaluation of the EMF group revealed morphological compromise and atrophy in the spinal cord, together with vacuolization in gray matter and occasional myelin thickening. Additionally, white matter infiltration was observed among nerve fibers, as well as marginal irregularity between white and gray matter, gray matter infiltration into white matter and motor neuron perikaryon injury. The histopathological findings from the present study are similar to those, and both studies show that 900 MHz EMF compromises the normal pathology of the spinal cord. However, these studies do not reveal whether the histopathological changes occurring under the effect of 900 MHz EMF are reversible. Further studies are needed to determine this.

Although it is impossible to make direct deductions concerning humans on the basis of the results of animal studies, the results of studies using rodents can be compared with humans at the same stage of development, irrespective of whether they are fetal, prenatal or postnatal (Rodier, 1980; Jacobson, 1991; Odacı et al., 2004). For example, the neonatal period of the CNS in rodents is comparable to the last trimester in humans (Dobbing, 1970; Dobbing and Sands, 1973; Rodier, 1980; Jacobson, 1991; Odacı et al., 2008). The study results may therefore be interpreted as meaning that prolonged and continuous exposure to 900 MHz EMF in the gestational period can compromise development of the human fetus spinal cord. In conclusion, our results show that continuous exposure to 900 MHz EMF for 1 h a day in early and middle adolescence causes degenerative changes in the rat spinal cord, such as atrophy, vacuolization, thickening and compromise in the myelin sheath, losses in axon cytoplasm and significant changes in MDA, SOD and CAT levels in spinal cord tissue.

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